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Passive dosing for the zebrafish embryo test using silicone O-rings

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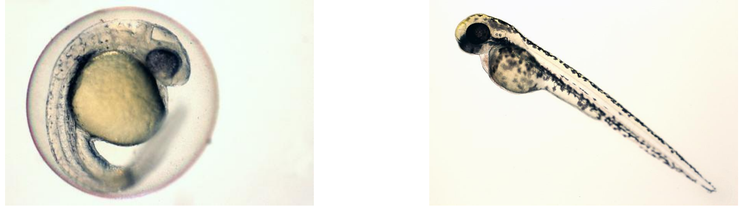
Background

Zebrafish Embryo Toxicity (ZFET) Test: OECD TG236

Hydrophobic organic compounds (HOCs) pose a challenge due to poorly defined exposure conditions.



Passive dosing
- Silicone O-rings
- Equilibrium partitioning

HOC(s) in methanol ⟷ 

Loading of O-rings

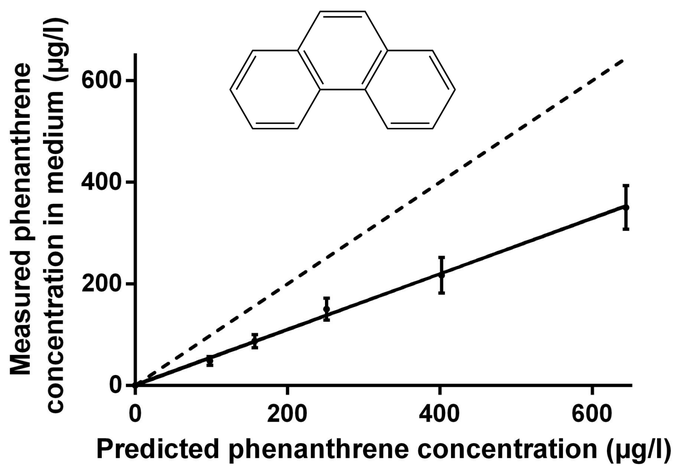
 ⟷ HOC(s) in test medium

Continuously replenish test medium

Loading principle:
Loading from a saturated methanol solution results in saturation of the test medium during exposure.
Loading from dilutions of the saturated methanol solution results in corresponding dilutions of the test medium.

Results & Discussion

Silicone O-rings produce a reliable and stable exposure concentration series

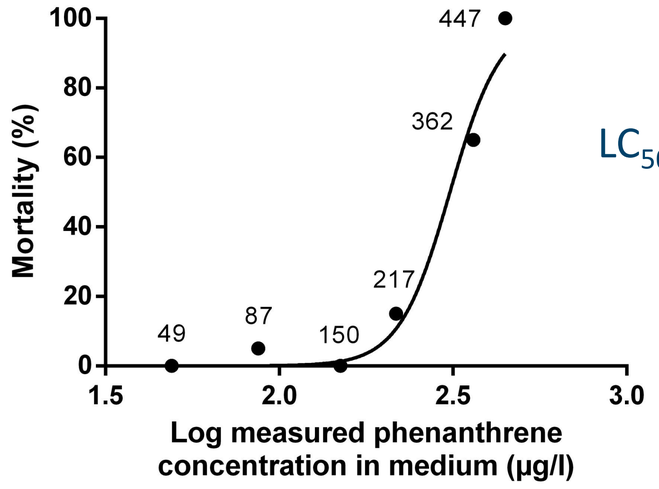


Y = (0.55 ± 0.01)x, n = 75, p < 0.0001 and R^2: 0.95

Reliable linear concentration series

SD < 10%

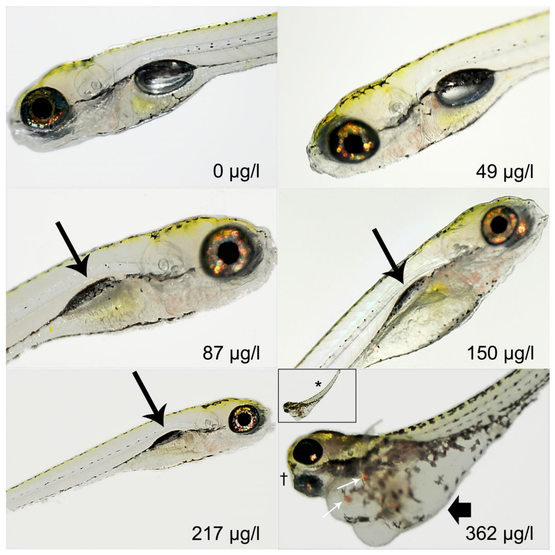
Controlled exposure concentrations facilitate precise determination of effect concentrations



Data labels indicate exposure concentration in µg/l

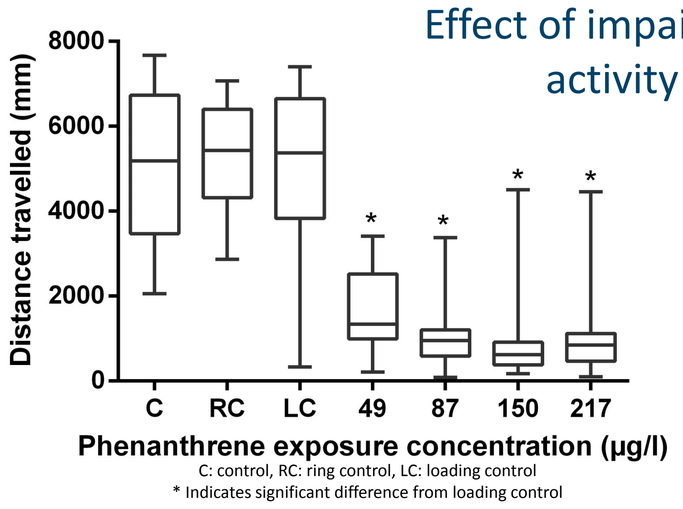
LC_{50} 120hpf phenanthrene: 310 µg/l (95%CI: 268 – 359)

Silicone O-rings are practical and allow for obtaining mechanistic toxicity information.



thin black arrow: swim bladder not inflated; thick black arrow: oedema of yolk (extension) and pericard; white arrow: blood accumulation in heart and yolk; †: malformation of eyes and mouth; asterisk in inset: curvature of the tail

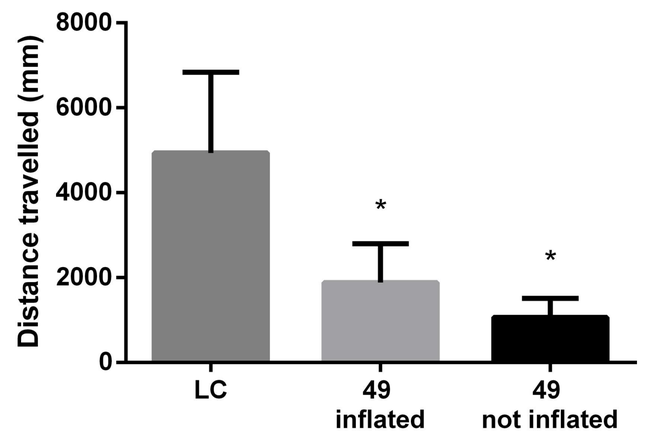
Impaired inflation of posterior swim bladder chamber is most important sublethal effect.



C: control, RC: ring control, LC: loading control
* Indicates significant difference from loading control

Swimming activity is reduced due to phenanthrene exposure.

Effect of impaired swim bladder inflation on swimming activity is small relative to narcosis effect.



LC: loading control
* Indicates significant difference from loading control

Advantages

- Reliable exposure when analytically confirmed
- Compatible with standard 24-well plate format
- Allows for individual follow-up
- Loading principle does not require prior knowledge of chemical partitioning behaviour

Practical considerations

- Cleaning and loading procedure time-consuming
- Testing at saturation would require loading at experimental temperature

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